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ZOÖLOGICAL BULLETIN.

FILOSE ACTIVITY IN METAZOAN EGGS.

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One who has not studied living Foraminifera or Radiolaria can get only an inadequate conception of the remarkable activities exhibited by the delicate protoplasmic extensions that form in such protozoans the thread-like pseudopodia. The figures and descriptions in text-books of zoölogy, in Verworn's *Physiology*, in Bütschli's *Protozoa*, or in special monographs naturally fall short of complete expression of the changeableness as well as the extreme delicacy of certain of these processes, though they teach us that the sensitive, contractile, coördinating powers of protoplasm may here be expressed in filaments of exceeding tenuity and inconstancy of form and position,— in flowing, liquid, apparently homogeneous protoplasm.

Such filose phenomena were practically unknown in Metazoa till a recent paper[1] described their occurrence in the eggs, polar bodies, blastulæ, gastrulæ, and larvæ of certain echinoderms. Here the cells put forth protoplasmic threads of excessive delicacy, that may branch and anastomose, elongate or shorten. By means of such filose processes the cells become connected amongst themselves, and, as these connections are living material comparable to the sensitive pseudopodia of many Protozoa, their importance in understanding the coördination of cells and their subordination to the entire mass, during the animals' development, was emphasized.[1]

Having been shown the filose threads in living starfish eggs, I have been able to observe the less attenuated ones present in

[1] Andrews, G. F., "Some Spinning Activities of Protoplasm in Starfish and Echinus Eggs," *Journ. of Morph.* Vol. xii. 1897.

the eggs of several other animals examined, and thus to extend the known occurrence of filose activities amongst Metazoa so widely that its importance seems strengthened and the probability of its still wider existence increased.

The first eggs examined, the frog's eggs in cleavage and gastrula stages, yielded when studied alive only the amœboid movement described by Roux; certain sections, however, showed intercellular connections that lead me to expect filose phenomena to be present here. A large number of sections of cleavage and larval stages in various frogs and in the salamander *Amblystoma punctatum* were carefully studied. In many sections of the latter animal, prepared and kindly loaned to me by Prof. C. B. Wilson of Westfield, Mass., as well as in certain frog's eggs, undoubted intercellular connections exist; but as their filose nature is not demonstrated, they will be but briefly noticed here. In the larva when the medullary folds are closed and the split mesoblast nearly fused on the ventral side, intercellular connections were seen between the large yolk cells, between mesoderm and mesenchyme cells, between the ectoderm cells on opposite sides of the nerve tube, between the ectoderm and mesoderm, and between the entoderm and mesoderm; in fact, cells in all germ layers and in each layer connect with those in another layer and with those in the same. Eliminating the deceitful appearances produced by coagulation of liquids between cells, by coagulation of fixative, by vacuolization and shrinkage of the superficial parts of cells, by the throwing off of pellicles, by the edges of drops and vesicles, and by fragments of vitelline membrane, as well as by scratches upon slide and cover glass, there still remained the above intercellular connections of undoubted protoplasm. These varied from fine filaments to broad bridges, and were either clear or contained some of the pigment granules of the egg. That they were filose in nature was indicated by their proportions and mode of origin and insertion; yet there was not decisive evidence that they were not produced in other ways either in the normal egg or in the egg when dying.

The next eggs, those of the annelid Serpula, were only examined alive and showed filaments passing out from the surface of the egg toward and to the membrane, both before and after the

first cleavage, as elsewhere mentioned.[1] Later, in the gastrula stage, filaments were seen passing from the ectoderm to the membrane. However, no undoubted case of filaments connecting cells was observed in the comparatively few eggs studied.

The eggs of a nudibranch mollusk, *Tergipes despectus* (?), were examined alive at a later date, and showed similar filose phenomena. In an egg not yet divided and having one polar body formed, numerous fine filose threads were seen projecting from the surface into the wide space between egg and membrane. Most of these filaments were confined to one quarter of the periphery, as seen in optical section; but one isolated, blunt, branched process came up some distance from the others near the polar bodies, which were of unequal lengths, a few longer ones reaching out halfway to the membrane. The longer ones often showed short branches at the tip and swellings along their length, suggesting those on the pseudopodia of filose Protozoa. Moreover, a vibrating particle beyond the finest filaments, and scarce seen with ocs. 6 and 8 and obj. 2 mm., moved out and then back again toward the egg, as if it might have been traveling upon some finer filament not seen.

The early stages of several marine lamellibranchs were very briefly examined. In *Yoldia limatula* an egg before dividing was seen to send out innumerable fine filaments from a thin layer of waving ectosarc. As these filaments were crowded together and radiated directly outward, they looked not unlike the cilia the larva developed, but they were much finer. These filaments did not spring from the entire surface of the egg, but from large areas. At one point a comparatively coarse process, suggesting an icicle in high refractive power and shape, projected amidst the finer filaments. As there was no membrane, all these filose processes projected freely into the sea water.

Again, in a much more prolonged study of the eggs of the large nemertean worm *Cerebratulus lacteus* Verrill, certain filose phenomena were seen before and after the first cleavage. The pear-shaped egg removed from the female had a more or less pronounced prolongation at its pointed pole. From this pro-

[1] Andrews, E. A., "Spinning in Serpula Eggs," *American Naturalist.* September, 1897.

longation fine filaments were seen to radiate in all directions and to rapidly change, and in other cases comparatively blunt filaments occupied the same place. Before this prolongation was drawn into the main mass, many fine filaments appeared from the opposite blunt end of the egg. As it was being drawn in, fine, short filaments were seen projecting from the surface of the egg round about its base. Under oc. 12, obj. 2 mm., these were much finer than the tail of the large sperms now often present within the egg membrane. When the polar bodies were forming, and for a time after their extrusion, the surface of the egg near these bodies (and sometimes quite generally) sent out very fine filaments, set like cilia close together.

Later, during the first cleavage, similar filaments arose from the surface of the egg. They were especially well seen when occurring as stout, stiff-looking, radiating lines arising from the tops of certain remarkable papillæ that frequently formed on the sides of the gaping cleavage furrow. As these small papillæ armed with tufts of filose processes arose at certain phases in cleavage and then vanished, they suggested some such temporary interconnection of cells as occurs in certain echinoderms;[1] but the filaments could not be followed from one cell to the other and seemed much too short to furnish any intercellular union by filose activity. In this connection it seems significant that the cleavage is of such a nature as to leave it doubtful, from surface views, whether the blastomeres actually separate entirely as in the echinoderms, or not.

In an egg with twenty or more cells fine processes were seen projecting from the profile of a cell favorably placed. In another case, where there were but four cells, a stout filament passed across the space between the inner ends of the cells, near the surface, and made the protoplasm of two opposite cells continuous. On this filamentous bridge there were nodular enlargements that gradually grew smaller as the filament dwindled in diameter and was withdrawn into one of the cells. But as the mode of formation of this connecting filament was not observed, and as the egg subsequently showed abnormal

[1] Andrews, G. F., "Some Spinning Activities of Protoplasm in Starfish and Echinus Eggs," *Journ. of Morph.* Vol. xii. 1897.

features, this was decided not to be a case of filose activity. In the same egg some shorter, slender pseudopodia projected from one cell, but could not be traced more than a tenth of the distance to the opposite cell toward which they extended over the above-mentioned polar space.

The most delicate filose displays were seen near the polar bodies during the first and second cleavages. The egg put forth fine protoplasmic threads that branched and reached up toward the second polar body. In this region a sheet of substance connected the egg with the second polar body, and the filose phenomena in it led to the assumption that it was a flowing mass of protoplasm, or that it contained more or less of it. But this, with the remarkable filose activities of the polar bodies, has been described and figured elsewhere.[1] In both a gasteropod and a lamellibranch, the polar bodies were likewise seen to have filose activities.[2] Thus in several great groups of animals the polar bodies may act in a filose way for some time after their extrusion, plainly exhibiting contractile phenomena in their cytoplasm and showing themselves to be still alive and active, so that, whatever may be their import as regards the chromatin they carry with them, they appear as more or less isolated parts of the egg mass, carrying on filose changes of the same nature as those of its other parts.

Being convinced that filose phenomena essentially similar to those of certain Protozoa exist also in the great metazoan groups Echinodermata, Annelida, Mollusca, and Nemertina, another attempt was made to find them in Chordata by studying some preserved Amphioxus eggs. However, the relation between living and preserved material is so remote in cases of such delicate phenomena as these sought, that little weight could be laid upon the results without a knowledge of the live eggs. Lacking this, it was thought that an acquaintance with the live and the preserved eggs of echinoderms would suffice to enable one to draw tentative conclusions from the preserved

[1] Andrews, E. A., "Activities of the Polar Bodies of Cerebratulus," *Archiv. f. Entwicklungsmechanik*. Bd. vi. 1898.

[2] Andrews, E. A., "Some Activities of Polar Bodies," *Johns Hopkins University Circulars*. Vol. xvii, No. 132. November, 1897.

Amphioxus eggs. The following three figures illustrate some of the appearances seen in live and in preserved echinoderm eggs, and may aid in justifying the conclusions formed as to the nature of the intercellular connections found in Amphioxus.

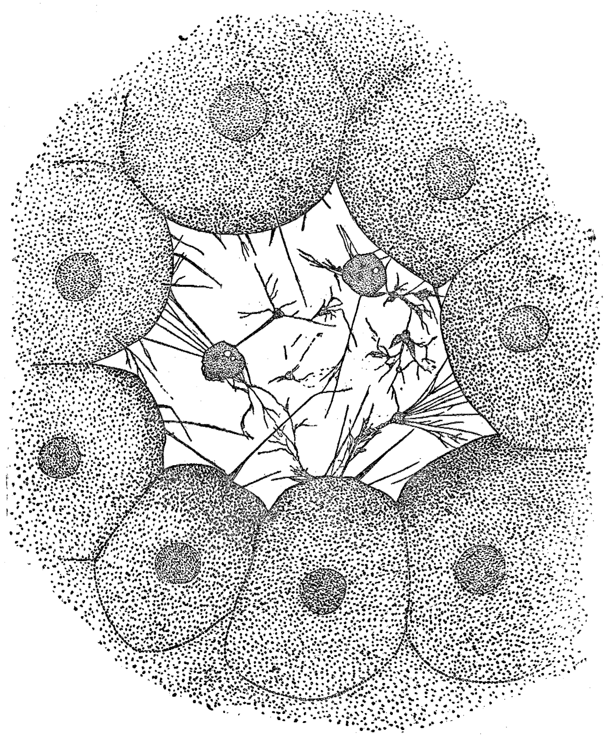

Fig. 1.

Fig. 1 represents a small part of a blastula of the starfish common at Roscoff, France, and is reduced one-half from a camera sketch made by G. F. Andrews in 1894 with oc. 8, obj. 2 mm., tube 170 mm. A small opening into the blastula is seen, surrounded by cells that are actively spinning out fine filaments by means of which they are variously connected with one another and with the two polar bodies. These lie, in this case, in the opening or cleavage pore, and are temporarily connected to adjacent cells while giving off very remarkable dendritic pseudopodia, along which protoplasm flows and collects in lumps. The chromosomes are not shown in the polar bodies,

nor in the enlargements on their pseudopodia, into which they were sometimes carried; vacuoles, however, are indicated in the main part of each polar body. The short, apparently blunt filaments projecting from the edge of the cell uppermost in the

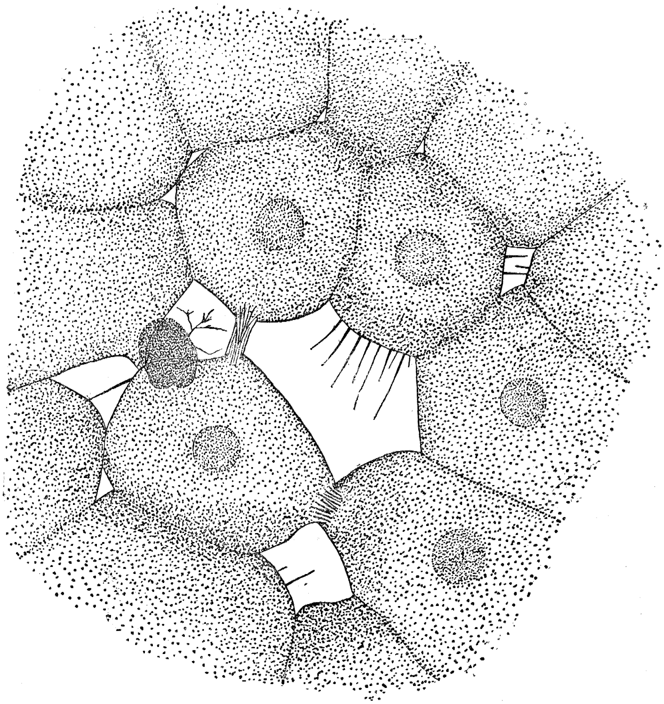

FIG. 2.

figure really represent threads that bent abruptly and extended far inward, and were, in most cases, attached to distant cells of the blastula.

This opening into the blastula closes in with change in position of the adjacent cells, and with changes in the character of the filose activities that lead one to conclude they are instrumental in associating the cells more intimately. Thus in Fig. 2 a later stage shows the cleavage pore reduced to a few small chinks between the cells that have glided over it. The polar bodies were here taken inside, and are represented, lying one over the other, by the black mass to the left.

In the lower part of the figure a cell to the left has reached out over one to the right and established a connection with it by means of a broad strand of filaments. An earlier stage of a similar process is shown above, where the same left cell is strongly bound to an upper cell by double strands of filaments. These filaments seem instrumental in drawing the cells together to cover in the cleavage pore. This figure is reduced one-half

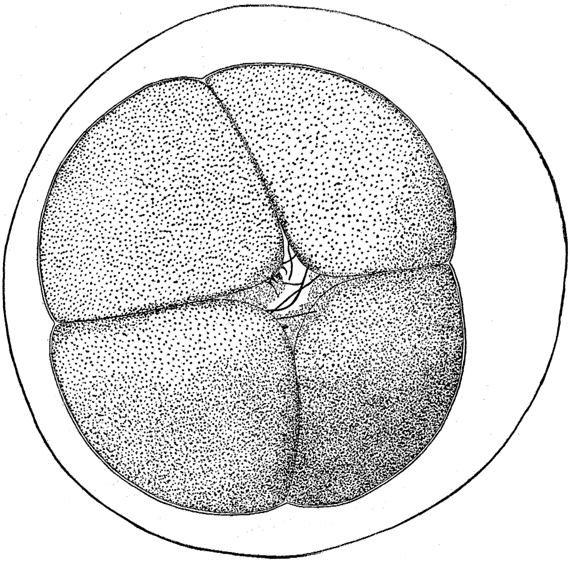

FIG. 3.

and otherwise like the first in execution, except that it was drawn with oc. 6.

In both these figures the thickness of the finer filaments is exaggerated in drawing, and hence they do not adequately indicate the delicacy of these processes. Moreover, as they are constantly changing, and as they contract and draw in when stimulated by certain chemicals or even by mechanical insult to the egg, it is plain that usual methods of preservation will fix but part of these displays, at the most, and that they may be readily broken off in subsequent treatment. However, it was found possible to preserve some of the larger filaments or amalgamations of filaments by special methods, and intercellular

connections and other spinnings have been retained, both in starfish and in sea-urchin eggs for three years.

Thus in Fig. 3 a four-cell stage in the common echinus of Roscoff shows filaments passing from cell to cell. These are drawn considerably thicker than the actual threads seen, but otherwise represent them truly. The figure is a surface view camera drawing with oc. 8, obj. 2 mm., tube 160 mm., helped

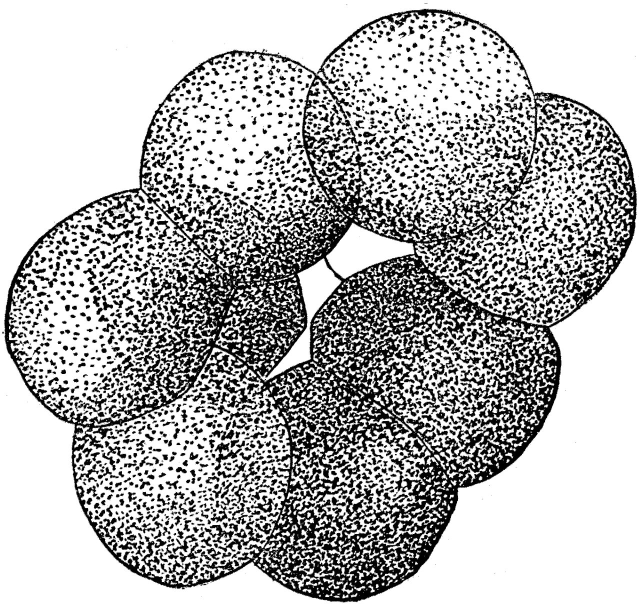

FIG. 4.

out with ocs. 12 and 18. On the left, above, a series of elevations from one cell seemed to be the remnants of tufts of filaments, while the granular matter, imperfectly represented here, partly covering the cleavage pore, appeared to be the same as Hammar's ectoplasmic layer.[1]

There can be no doubt that these filamentous intercellular connections are the preserved remnants of the active filose threads of the living egg.

Some eggs of Amphioxus killed in corrosive acetic and stained in Orth's new lithium carmine, as well as some killed

[1] Andrews, E. A., "Hammar's Ectoplasmic Layer," *American Naturalist.* December, 1897.

in Flemming's fluid and not stained, were kindly placed at my disposal by Professor T. H. Morgan. Both those mounted in balsam and those studied in alcohol showed undoubted filaments connecting the blastomeres in various cleavage stages. From the resemblance of these filaments to those found in preserved echinoderm material there seemed little doubt that this was probably another case of filose intercellular connections, but there must remain some doubt till the live egg is studied. In living material we may expect to find filose displays as remarkable as those in the echinoderms, and, in part at least, more readily observed.

In four, eight, and sixteen-cell stages, many eggs showed such marked intercellular connections as the one represented in Fig. 4. These filaments are of clear material that arises from the clear ectosarc of one cell and becomes continuous with that of another cell. Only a few cases of branching were seen, apparently only the main trunks and grosser threads being preserved.

Fig. 4 shows an eight-cell stage in Amphioxus, with a definite abruptly curved filament passing from one cell to an opposite one at the bottom of an open cleavage cavity. This is from a camera sketch with oc. 2 and obj. D, not reduced. One cell showed a marked elongation toward another, but no connecting filaments were seen, though they may well have existed there in the live state. Besides the filaments seen connecting cells, as in the above figure, there were other signs of filose activity in these eggs; groups of minute spherules and filaments protruded from the ectosarc as if remnants of filose processes. There were also large ectosarcal outflows near cleavage planes, suggesting the amœboid elevations described for the eggs of certain nematodes by Erlanger.[1]

When more highly magnified these intercellular filaments in Amphioxus appear as in Fig. 5, which represents part of another eight-cell stage, drawn with camera, oc. 8 and obj. $\frac{1}{12}$ im. Here a filament arose from the ectosarc of one cell, and, after making a complex bend, difficult to understand, gradually dwindled in diameter till it became continuous with the surface of another

[1] *Biol. Centrblt.* Bd. xvii. 1897.

cell on the opposite side of a narrow cleavage space at the center of the rather compact group of eight cells.

Such intercellular connections as this would seem to be, in all probability, of the same nature as those seen in live and in preserved echinoderm eggs. And if such be the case, it is especially interesting to find them in Amphioxus, not only as this is in many respects so diagrammatic a representative of the Chordata as to lead one to infer its filose phenomena will

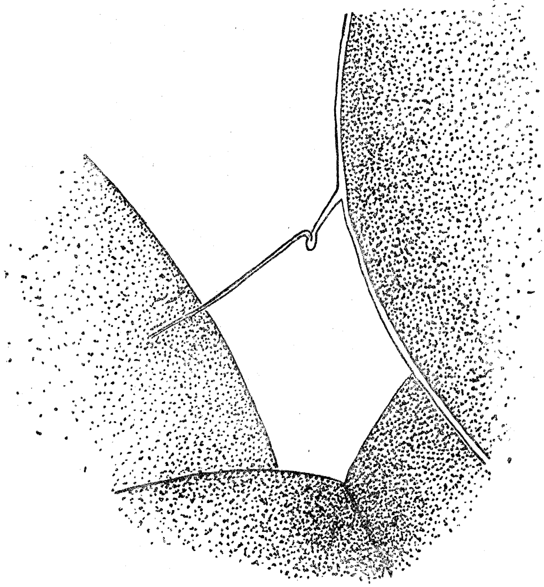

Fig. 5.

be found, in modified form, in various vertebrates, but because the egg of Amphioxus has been so carefully studied by experimental methods that the need of an organic intercellular connection to explain known facts in embryology is here especially felt. Thus Prof. E. B. Wilson was led to conclude in his study of Amphioxus,[1] "*that the unity of the normal embryo is not caused by a mere juxtaposition of the cells. . . . This unity is not mechanical, but physiological. . . . There must be a struc-*

[1] "Amphioxus and the Mosaic Theory of Development," *Journ. of Morph.* Vol. viii. 1893.

tural continuity from cell to cell that is the medium of coördination, and that is broken by mechanical displacements of the blastomeres."

The nature of this structural continuity was not surmised, but it now seems evident that it is really, in part at least, brought about by such remarkable, changing, pseudopodial threads as were seen in the echinoderms and preserved, in part, in the Amphioxus eggs I had for examination.

That filose phenomena will be found in the blastula and gastrula stages seems most probable, but as yet I have not been able to find remnants of them in the above-preserved material. The figures published by Klaatsch [1] in illustration of other problems suggest, at first sight, profuse intercellular connections in these stages of Amphioxus, but it seems more probable that the lines there shown are the results of shrinkage and imperfect preservation, though some filose activity may have furnished an element for certain of the distortions that resulted.

As filose phenomena in eggs as far as yet studied are, to say the least, easily overlooked (in the echinoderms, they can be seen only with difficulty, though the main threads from the polar bodies of Cerebratulus and the above connections in Amphioxus are so distinct as to be readily evident with low powers to one searching for them), we need not expect to find them frequently mentioned in the past literature of embryology. Yet some of them must have been seen, even if passed by as of little moment. Thus Professor Conn described and figured [2] fine lines passing out from the surface of the egg of the gephyrean worm *Thalassema mellita* Conn to the rather distant membrane. These he regarded as striæ and interpreted as indicative of the presence of a jelly-like substance between egg and membrane. Yet an examination of his Fig. 13, Pl. XX, suggests that this egg and its polar bodies will prove to possess filose phenomena.[3]

[1] "Bemerkungen über die Gastrula des Amphioxus," *Morph. Jahrb.* Bd. xxv, 1897. Pl. XII.

[2] Studies from the Biological Laboratory, Johns Hopkins University. Vol. ii. 1884.

[3] In correcting proof I add that the connection made by Professor Flemming (*Merkel and Bonnet, Ergebnisse*, 1897, p. 279) between certain fine pseudopodia-

Conclusions.

Filose activities like those of the finer pseudopodial threads of certain Protozoa were seen in the living eggs of Metazoa, in Echinodermata, Annelida, Mollusca, and Nemertina. Study of preserved material makes most probable their existence in Amphioxus and quite probable their existence in Amphibia. Members of other great groups have not as yet been examined from this point of view.[1]

Such filose filaments connect the cells in the eggs and larvæ of Echinodermata; filaments that are most probably of this nature connect the blastomeres of Amphioxus; filaments probably filose connect the cells in eggs and larvæ of Amphibia.

Wherever found, filose connecting filaments may be assumed to have the importance ascribed to them on their first discovery in the echinoderms, and to furnish a medium for coördinating the activities of parts of the embryo.

Johns Hopkins University, Baltimore,
January 29, 1898.

like lines seen by him on a polar body of Anodonta in 1873 (*Archiv. f. mikr. Anat.*, Bd. x) and the filose phenomena of Echinodermata as described by G. F. Andrews seems to me probably correct.

[1] However, at this later date, March 7, I am able to state that the cleaving eggs of a green Hydra have remarkable ectosarcal displays and some interconnection of cells by filose activities; this in addition to the gross pseudopodia described by Kleinenberg.